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Date: 2/26/2002
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Inventor Name Search Result

Your Search was:

Last Name = BRAIS
First Name = BERNARD

Application#	Patent#	Status	Date Filed	Title	Inventor Name
09590211	Not Issued	071	06/08/2000	SHORT GCG EXPANSIONS IN THE PAB II GENE FOR OCULO-PHARYNEAL MUSCULAR DYSTROPHY AND DIAGNOSTIC THEREOF	BRAIS, BERNARD
60152941	Not Issued	159	09/09/1999	DIAGNOSIS, PROGNOSIS AND TREATMENT OF REPEAT-ASSOCIATED DISEASES AND INTRANUCLEAR INCLUSIONS-ASSOCIATED DISEASESE	BRAIS , BERNARD

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Inventor Name Search Result

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Last Name = ROULEAU
First Name = GUY

Application#	Patent#	Status	Date Filed	Title	Inventor Name
09508821	Not Issued	041	05/26/2000	POLYMORPHIC CAG REPEAT-CONTAINING GENE AND USES THEREOF	ROULEAU, GUY
09509312	Not Issued	041	06/09/2000	ALLELIC VARIANTS OF THE PHOSPHOLIPASE C-YAMMA1 GENE ASSOCIATED WITH BIPOLAR DISORDER	ROULEAU, GUY
09590211	Not Issued	071	06/08/2000	SHORT GCG EXPANSIONS IN THE PAB II GENE FOR OCULO-PHARYNEAL MUSCULAR DYSTROPHY AND DIAGNOSTIC THEREOF	ROULEAU, GUY
09718355	Not Issued	019	11/24/2000	LOCI FOR IDIOPATHIC GENERALIZED EPILEPSY, MUTATIONS THEREOF AND METHOD USING SAME TO ASSESS, DIAGNOSE, PROGNOSE OR TREAT EPILEPSY	ROULEAU, GUY
09830891	Not Issued	040	08/06/2001	POLYGLUTAMINE-CONTAINING PROTEINS IN NEUROPSYCHIATRIC DISORDERS	ROULEAU, GUY
60152941	Not Issued	159	09/09/1999	DIAGNOSIS, PROGNOSIS AND TREATMENT OF REPEAT-ASSOCIATED DISEASES AND INTRANUCLEAR INCLUSIONS-ASSOCIATED DISEASESE	ROULEAU , GUY A.
60167623	Not Issued	159	11/26/1999	LOCI FOR IDIOPATHIC GENERALIZED EPILEPSY, MAPPING TO CHROMOSOME 2, MUTATIONS THEREOF AND METHOD USING SAME TO ASSESS, DIAGNOSE, PROGNOSE OR TREAT EPILEPSY	ROULEAU , GUY A.

60287035	Not Issued	020	04/30/2001	LOCI FOR IDIOPATHIC GENERALIZED EPILEPSY, MUTATIONS THEREOF AND METHOD USING SAMETO ASSESS, DIAGNOSE, PROGNOSE OR TREAT EPILEPSY	ROULEAU, GUY
60295168	Not Issued	020	06/01/2001	GENE-BASED ASSAYS FOR THERAPEUTIC AGENTS USEFUL IN TREATING NEUROLOGICAL DISORDERS	ROULEAU, GUY

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(FILE 'HOME' ENTERED AT 11:09:18 ON 26 FEB 2002)

FILE 'CAPLUS, BIOSIS, EMBASE, MEDLINE' ENTERED AT 11:09:30 ON 26 FEB 2002

L1	1058	S (PAB OR PABII OF PAB2) (L) (REPEAT OR SEQUENCE OR HUMAN)
		E POLYA
L2	198	S E3 (L)(BINDING (L) PROTEIN)
L3	9	S L2 AND (PAB OR PAB2 OR PABII)
L4	3786	S L2 OR (PAB OR PAB2 OR PABII)
L5	57	S L4 (L) (REPEAT OR CPG)
L6	517	S L4 (L) (REPEAT OR CPG OR MUTATION OR ALTERATION)
L7	200	DUPLICATE REMOVE L6 (317 DUPLICATES REMOVED)
L8	140	S L7 AND HUMAN
L9	3	S L8 AND CPG

Restriction map of a YAC and cosmid contig encompassing the oculopharyngeal muscular dystrophy candidate region on chromosome 14q11.2-q13.

Xie YG, Rochefort D, Brais B, Howard H, Han FY, Gou LP, Maciel P, The BT, Larsson C, Rouleau GA.

Center for Research in Neuroscience, McGill University, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec, H3G 1A4, Canada.

As part of our effort to clone positionally the oculopharyngeal muscular dystrophy (OPMD) gene, we constructed a YAC contig, a cosmid contig, and an EcoRI restriction map of the OPMD candidate region. The YAC contig spans more than 2 Mb and encompasses the loci D14S283 and D14S990 and the cardiac alpha and beta myosin heavy chain genes (MYH6 and MYH7). A 700-kb cosmid contig containing the D14S990 and the myosin genes and a long-range restriction map covering the region between D14S990 and the MYH6 and MYH7 gene cluster were established. A detailed EcoRI restriction map of the cosmid contig was determined, and five putative CpG islands were identified. Based on these data, the four loci were mapped within an approximately 600-kb region with the following centromere to telomere order: D14S283, D14S990, MYH6, and MYH7. The YAC and cosmid contigs will facilitate the identification of genes lying within the OPMD candidate interval.

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SHORT COMMUNICATION

Restriction Map of a YAC and Cosmid Contig Encompassing the Oculopharyngeal Muscular Dystrophy Candidate Region on Chromosome 14q11.2–q13

Ya-Gang Xie, Daniel Rochefort, Bernard Brais, Heidi Howard, Fei-Yu Han, Lu-Ping Gou, Patricia Maciel, Bin Tean The,* Catherina Larsson,* and Guy A. Rouleau[1]

*Center for Research in Neuroscience, McGill University, and Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada; and * Department of Molecular Medicine, Clinical Genetics Unit, Karolinska Hospital, L-6 Building, S-171 76 Stockholm, Sweden*

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As part of our effort to clone positionally the oculopharyngeal muscular dystrophy (OPMD) gene, we constructed a YAC contig, a cosmid contig, and an *Eco*RI restriction map of the OPMD candidate region. The YAC contig spans more than 2 Mb and encompasses the loci D14S283 and D14S990 and the cardiac α and β myosin heavy chain genes (MYH6 and MYH7). A 700-kb cosmid contig containing the D14S990 and the myosin genes and a long-range restriction map covering the region between D14S990 and the MYH6 and MYH7 gene cluster were established. A detailed *Eco*RI restriction map of the cosmid contig was determined, and five putative CpG islands were identified. Based on these data, the four loci were mapped within an approximately 600-kb region with the following centrom re to telomere order: D14S283, D14S990, MYH6, and MYH7. The YAC and cosmid contigs will facilitate the identification of genes lying within the OPMD candidate interval.

Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant disease characterized by late onset (usually after the age of 50) progressive drooping of eyelids (ptosis) and difficulty swallowing (dysphagia) (11). Based on a linkage study of French Canadian families with OPMD, we have previously mapped the OPMD locus to a 3.6-cM interval between marker D14S50 and two intronic $(CA)_n$ markers from the cardiac β myosin heavy chain gene (D14S1457 and D14S1458) on human chromosome 14q11.2–q13 (3). This interval has been further reduced to 2 cM between D14S283 and two cardiac β heavy chain gene markers (4). To characterize the OPMD candidate region, determine the chromosomal orientation of MYH6 and MYH7, and identify genes in this region, we have established a physical map containing the loci D14S283, D14S990, MYH6, and MYH7, consisting of a 2-Mb YAC contig and a cosmid contig with an *Eco*RI restriction map that covers a total of 700 kb.

Probes for D14S283 (124–152 bp) and D14S990 (125–153 bp) were generated by PCR amplification using published primer sequences (5). Probes E35M6 (253 bp) and E4M7 (254 bp) are the PCR products of exon 35 of MYH6 and exon 4 of MYH7, respectively (6, 8), amplified using intronic primers E36M6-A (5′TGGAGAAAGGGTATGAAATCAGGT3′) and E36M6-B (5′CCCAAGGCCTTGTTTCTGTCTTTA3′); E4M7-A (5′TTGAGGAAGGAGGGGAAGC3′) and E4M7-B (5′CATGGATGGAGCAAGAACAGAGAT3′). Primers for D14S990 and D14S283 (S990-A and S283-A), E35M6-A, and E4M7-A (described above) and intronic primers for exon 1 of MYH6 (E1M6-A: 5′GGGGAAACGGGATATAAAGGAAC3′), exon 39 of MYH6 (E39M6-B: 5′GCCCCCTACTGCCCTGAT3′), exon 1 of MYH7 (E1M7-A: 5′GTGACAACAGCCCTTTCTAAATCC3′), and exon 40 of MYH7 (E40M7-B: 5′ATTGCTTTATTCTGCTTCCTC3′) were used as oligonucleotide probes.

D14S283, D14S990, E4M7, and E36M6 were used as start points in the construction of the genomic contigs. YAC 26a9 was identified by PCR screening of a human genomic YAC library (1) with markers E35M6 and E4M7, and YACs 806b10 and 926c7 were identified for the D14S990 locus. Three YACs containing locus D14S283 were selected from the Genome Data Base (GDB). All YAC clones were obtained from the Canadian Genetic Diseases Network (CGDN) DNA Core Facility. The six YACs were characterized by Southern analysis of PFGE-separated YAC blots using *Eco*RI fragments from cosmids containing D14S283, D14S990, MYH6, and MYH7. The sizes of YACs were estimated to be 460 (26A9), 600 (926c7), 750 (806b10), and 790 kb (765d11). The sizes of YACs 799a10 (1780 kb) and 980g9 (1600 kb) were obtained from the GDB. YAC 26a9 was confirmed to contain both MYH6 and MYH7, and YACs 806b10, 765d11, and 980g9 contain the MYH6 and MYH7

[1] To whom correspondence should be addressed at Department of Neurology, Montreal General Hospital, L7-224, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4, Canada. Telephone: (514) 937-6011, ext. 4235. Fax: (514) 934-8265. E-mail: mi32@musica.mcgill.ca.

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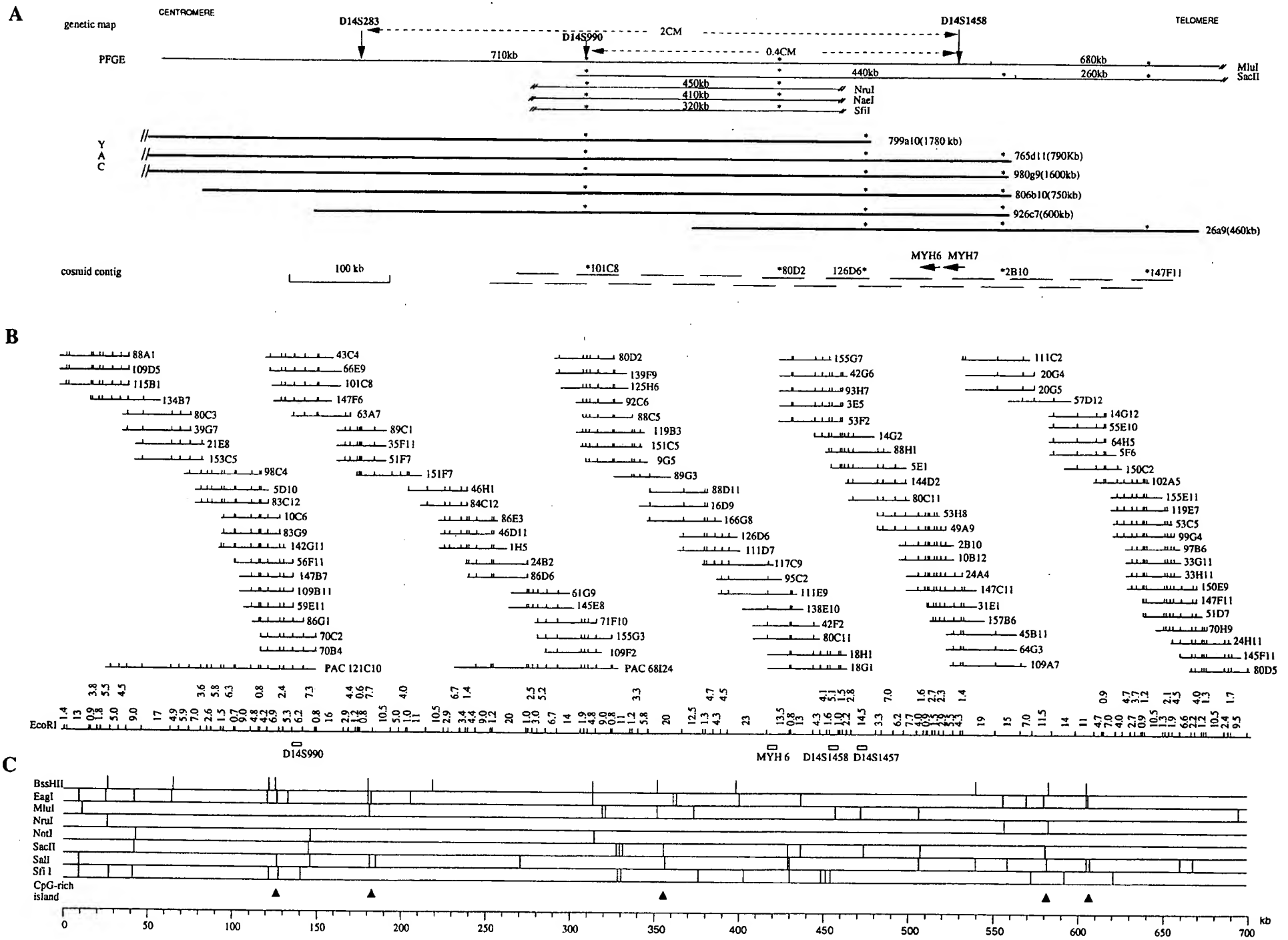
A
genetic map
CENTROMERE
D14S283
2CM
D14S990
D14S1458
TELOMERE
PFGE
710kb
0.4CM
680kb
MluI
440kb
260kb
SacII
450kb
NruI
410kb
NaeI
320kb
SfiI
YAC
799a10(1780 kb)
765d11(790Kb)
980g9(1600kb)
806b10(750kb)
926c7(600kb)
26a9(460kb)
MYH6 MYH7
cosmid contig
100 kb
*101C8
*80D2
126D6*
*2B10
*147F11
B
88A1
109D5
115B1
134B7
80C3
39G7
21E8
153C5
98C4
5D10
83C12
10C6
83G9
142G11
56F11
147B7
109B11
59E11
86G1
70C2
70B4
PAC 121C10
43C4
66E9
101C8
147F6
63A7
89C1
35F11
51F7
151F7
46H1
84C12
86E3
46D11
1H5
24B2
86D6
61G9
145E8
71F10
155G3
109F2
PAC 68I24
80D2
139F9
125H6
92C6
88C5
119B3
151C5
9G5
89G3
88D11
16D9
166G8
126D6
111D7
117C9
95C2
111E9
138E10
42F2
80C11
18H1
18G1
155G7
42G6
93H7
3E5
53F2
14G2
88H1
5E1
144D2
80C11
53H8
49A9
2B10
10B12
24A4
147C11
31E1
157B6
45B11
64G3
109A7
111C2
20G4
20G5
57D12
14G12
55E10
64H5
5F6
150C2
102A5
155E11
119E7
53C5
99G4
97B6
33G11
33H11
150E9
147F11
51D7
70H9
24H11
145F11
80D5
EcoRI
D14S990
MYH6
D14S1458
D14S1457
C
BssHII
EagI
MluI
NruI
NotI
SacII
SalI
Sfi I
CpG-rich island
0
50
100
150
200
250
300
350
400
450
500
550
600
650
700
kb

gene cluster, D14S990 and D14S283. Only D14S990 and D14S283 detected YACs 799a10. These results allowed us to contig all the YACs, except YAC 26a9, and map loci D14S283 and D14S990 to a 600-kb region.

In parallel, the Los Alamos gridded chromosome 14-specific cosmid library, constructed from flow-sorted chromosomes cloned into vector scos-1 and consisting of approximately 16,000 recombinants, was used to isolate 17 cosmids using the MYH6, MYH7, E35M6, and E4M7 probes. The cosmid library was screened by direct hybridization to 14 gridded filters, each containing 1152 clones, constructed within the laboratory and processed as described in Lafrenière *et al.* (9). Based on the occurrence of same-sized *Eco*RI fragments, these cosmids were assembled into a contig spanning 96 kb. Hybridizing E35M6 and E4M7 mapped MYH6 and MYH7 back to the contig to *Eco*RI Southern blots of the cosmids. Using a similar strategy, we identified and characterized three cosmids corresponding to loci D14S283 and 12 cosmids for D14S990.

Simultaneously, *Alu*-PCR amplified products from YAC 26a9 were used to probe the cosmid library. Nineteen cosmids were identified, some of which had already been assigned to the cosmid contig containing the MYH genes. The cosmids that did not map to the previously identified contigs were used to probe the cosmid library. This resulted in expansion of the contig containing MYH genes in both directions and construction of several independent short contigs ranging in size from 60 to 80 kb.

Cosmid contigs were constructed by bidirectional walking from the MYH6 and MYH7 gene cluster, D14S283 and D14S990, as described in Lafrenière *et al.* (9). As chromosome walking progressed, we encountered a low "clonal represented region." Single-copy *Eco*RI fragments from cosmid 153C5 (1.2 kb) and cosmid 155G3 (2.0 kb) were used to screen a total human PAC library (RPCI-1; information on the library is available at http://bacpac.med.buffalo.edu; Ref. 7). PAC 68I24 and PAC 121C10 were identified. PAC 68I24 helped us to anchor the region containing cosmid 71F10 and cosmid 86D6, resulting in all the cosmids becoming part of a large contig. The contig is composed of 110 cosmids starting from D14S990 and the MYH6 and MYH7 genes and spans 700 kb (Fig. 1). The PAC clones were obtained from the CGDN DNA Core Facility.

The *Eco*RI restriction map of the contig was constructed by identifying shared restriction fragments in overlapping clones, as described in Lafrenière *et al.* (9). It yields an estimated span of 700 kb centered between the D14S990 locus and the MYH6 and MYH7 genes (Fig. 1B). Based on the previously reported genetic maps placing D14S283 centromeric to D14S990 and MYH (5), and the orientation of the cosmid contig within the YAC contig, we established that MYH6 is centromeric to MYH7 (Fig. 1). The MYH6 and MYH7 genes were placed on our *Eco*RI restriction map using oligo probes from both ends of the two genes (E1M6-A, E39M6-B, E1M7-A, E40M7-B) (Fig. 1A).

To characterize further the OPMD candidate region, we performed PFGE mapping, as described in Ruttledge *et al.* (10), with three DNA markers chosen from the cosmid contig. These *Eco*RI fragments were isolated from near the MYH6 and MYH7 genes (80D2 and 147F11) or from the cosmids containing locus D14S990 (101C8). A 4.8-kb *Eco*RI fragment from 80D2, a 2-kb *Eco*RI fragment from 147F11, and a 5.3-kb *Eco*RI fragment from the D14S990 cosmid (101C8) were used to probe several PFGE Southern filters individually. Results show that cosmids 80D2 and D14S990 cohybridize to a 710-kb *Mlu*I fragment, a 410-kb *Nae*I fragment, a 450-kb *Nru*I fragment, a 440-*Sac*II fragment, and a 320-*Sfi*I fragment. The probe from cosmid 147F11 identified only a 680-kb *Mlu*I and a 260-kb *Sac*II fragment, but failed to recognize any bands from DNA digested with other enzymes. These results confirmed the YAC contig, the chromosomal orientation of the cosmid contig, and the chromosomal orientation of MYH6 and MYH7 (Fig. 1A).

Based on the definition of a CpG-rich island as a clustering of four or more of the following eight restriction enzymes, *Bss*HII, *Eag*I, *Mlu*I, *Nru*I, *Not*I, *Sac*II, *Sal*I, and *Sfi*I, within a 2-kb interval, we could identify five separate CpG-rich islands dispersed throughout the contig (Fig. 1C; Refs. 2, 9).

We report a detailed physical map of the OPMD candidate region, which includes a YAC contig and a cosmid contig, as well as both a long-range and an

FIG. 1. YAC and cosmid contig of the OPMD candidate region on 14q11.2–q13. (A) A genomic contig with a long-range restriction map: the approximately 2-Mb YAC contig consists of six overlapping YAC clones, depicted by long horizontal boldface lines. The names and approximate sizes of the YACs are shown on the right. The location of the cosmid contig is shown. Each cosmid is indicated by a short horizontal line that represents approximately 40 kb. The contig spans approximately 700 kb. The arrows represent the location, genomic size, and transcriptional orientation of the MYH7 and MYH6 genes. The centromere is to the left, and the telomere is to the right. The sizes and location of *Mlu*I, *Nae*I, *Nar*I, *Sac*II, and *Sfi*I restriction fragments are shown above the YAC contig. The cosmids used as probes to characterize the YAC contig and PFGE mapping are indicated by an asterisk. YACs and pulse-field fragments labeled with an asterisk have been identified by the cosmid marked with an asterisk shown below them. A summary of genetic mapping results for markers D14S283, D14S990, and D14S1458 is also shown at the top. The genetic distances from locus D14S1458 to D14S990 and D14S283 are given. (B) Details of cosmid and PAC contigs with *Eco*RI restriction map. Relative overlaps of 110 cosmids and two PACs that include MYH6, MYH7 (D14S1457 and D14S1458), and locus D14S990 are shown; the locations of these loci are shown as rectangles beneath the restriction map. Overlaps are based on sharing of common-size *Eco*RI fragments (vertical lines in each clone). Clones are designated by their coordinates in the gridded library. Consensus *Eco*RI restriction map of the contig is based on the cosmid overlaps. Numbers above the maps correspond to the sizes of *Eco*RI fragments in kb. (C) Restriction map of *Bss*HII, *Eag*I, *Mlu*I, *Nru*I, *Not*I, *Sac*II, *Sal*I, and *Sfi*I relative to the *Eco*RI map. Putative CpG islands are indicated as dark arrowheads.

*Eco*RI restriction map. The YAC contig is composed of six overlapping YACs, spans a distance of more than 2 Mb, and contains loci D14S283, D14S990, MYH6, and MYH7. The cosmid contig within this region spans approximately 700 kb. A long range PFGE restriction map covers the region between D14S990 and the MYH6 and MYH7 gene cluster, and an *Eco*RI restriction map has been established for the entire cosmid contig. We have mapped D14S283, D14S990, MYH6, and MYH7 to an approximately 600-kb region and determined the order and the chromosomal orientation of these markers (from centromere to telomere—D14S283, D14S990, MYH6, and MYH7).

We have found that establishing a restriction map simultaneously with the construction of a cosmid contig facilitates chromosome walking. This practice helps identify the most extreme end-cosmid, confirms the overlaps, and helps exclude the possibility of false-positive links caused by cross-hybridization or rearranged clones.

Our physical map and genomic contig will also help to elucidate this subchromosomal region, to map and characterize known genes, and to help identify novel genes in this region. The cosmid contig will also facilitate the sequencing of chromosome 14.

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